COLD DEATH IN THE GUPPY [1]

RONALD B. PITKOW [2]

Department of Physiology, School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania

There are numerous reports describing the death of vast numbers of fish subsequent to cold waves, often where the water temperature remained well above 0° C. (Wilcox, 1887; Verrill, 1901; Vatova, 1929; Storey and Gudger, 1936; Storey, 1937; Miller, 1940; Gunter, 1941; Gunter, 1947; Mosevich, 1944).

Maurel and Lagriffe (1899) divided the changes induced by placing fish into colder and colder water into five stages. Stage five consisted of convulsions, loss of equilibrium, complete paralysis, and a state of apparent death; the fish would revive only if warmed within a few minutes. If not warmed, they would go on to die without showing any visible signs of life. Doudoroff (1942) called this "primary chill coma."

At less extreme temperatures a different sequence of events occurred. The fish would recover from the initial shock, resume respiration, if it had stopped, and take on a relatively normal appearance. If kept at such a temperature for some hours or days, the fish would show increasing distress and finally cease to respond to stimuli and to respire. This characteristic response to less extreme temperatures, Doudoroff (1942) called "secondary chill coma." In "primary chill coma" the fish die without a return of vital signs, unless rewarmed; in "secondary chill coma," vital signs, if they disappear, return for a time and the fish then gradually die unless rewarmed.

Virtually nothing is known of the mechanism of death due to "primary chill coma." Brett (1952) experimented with cold tolerance in young Pacific salmon and concluded that death occurring in the first hour of a cold exposure was probably due to a disturbance of the central nervous system. It has been shown that with decreased acclimation temperature a lower temperature is required to induce "primary chill coma" (Samochvalova, 1938) and the fish can withstand longer exposures to a lethal cold temperature (Fry, Brett and Clawson, 1942; Doudoroff, 1942; Brett, 1956).

Only Doudoroff (1945) has proposed a mechanism of death due to "secondary chill coma." He showed that the death of *Fundulus*, a salt-water fish, at slowly lethal temperatures in sea water was preceded by osmotic dehydration of tissues, and was delayed in diluted sea water. He concluded that osmoregulative failure was one of the causes of slow death in cold sea water.

The characteristics of cold tolerance in the guppy, *Lebistes reticulatus,* have never been thoroughly explored. The guppy is an ideal subject for inquiry into

[1] Supported by a National Foundation for Infantile Paralysis Research Fellowship, and Public Health Service Training Grants to the author, and by a National Science Foundation grant to Dr. John R. Brobeck.

[2] Present address: Abington Memorial Hospital, Abington, Pennsylvania.

the effects of cold on fish. It is hardy, inexpensive, and easily procured. More than 4500 guppies were used in the past three and one-half years to secure the information presented in this paper.

This paper will propose for the first time a possible mechanism of death due to "primary chill coma" in fish.

Methods and Material

Approximately 1000 guppies were obtained from aquatic plant tubs in the greenhouses of the University of Pennsylvania, Department of Botany. The remainder were bought from Florida fish farms. The fish were acclimated for two weeks to five-, ten-, or fifteen-gallon stock aquaria, with up to 40 fish per gallon of water. The fish were fed various commercial brands of dry fish food and Dash dog food once daily.

Randomly chosen fish were taken from stock aquaria by means of a soft net and quickly placed into the test water which was maintained at uniform temperature (0.1° C.) by gentle shaking of the container unless otherwise stated, by the gradual addition of ice to a constant temperature bath into which the test container

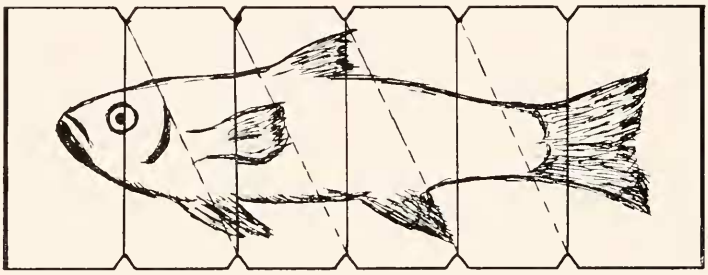

Figure 1. Comatose fish secured to a notched glass slide with a soft cotton thread.

was immersed. Temperatures were read from standardized mercury thermometers graduated to 0.1° C. or 0.05° C. At the completion of a test exposure or a series of stepwise test exposures the fish were quickly removed and placed in water from the stock tank.

Once a fish regained normal respiration and was able to swim normally after a test exposure, it almost invariably lived. Those fish alive 24 hours after test exposures were considered as having survived the insult. Deaths after this 24-hour period were few, always less than 5%, and seemed to depend mostly on the general condition of the fish prior to the cold exposure.

In the experiments in which fish were subjected to cold in oxygen-depleted water or oxygen-enriched water, the water was vigorously bubbled and equilibrated with 100% nitrogen or 100% oxygen for one hour immediately prior to its use. In approximately one-half of these experiments a sample of test water was siphoned directly into a sampling jar at the midpoint of the cold exposure and immediately subjected to a Winkler oxygen determination. In all the experiments where a Winkler oxygen determination was performed, distilled water was used in the

test container, rather than stock tank water, so as to minimize the amount of organic material which might interfere with the accuracy of the oxygen determination. The substitution of distilled water for stock tank water did not alter the results. In those experiments where an oxygen determination was not performed, the oxygen content was estimated and rounded off to the nearest 5 mg./L.

Some of the data to be presented are observations on individual fish of the relationship of respiration to the presence of a functioning circulation. Gill and mouth respiratory movements were easily seen with the naked eye. The criterion for the presence of a functioning circulation was the presence of blood flow in the arteries and veins at the base of the fish's tail. Thirty-five or 100 diameters magnification of these vessels rendered blood flow clearly visible. A fish after being paralyzed in the cold test water was quickly placed on a notched glass slide and gently secured with soft cotton thread as diagrammed in Figure 1. This necessitated removing the fish from the test water for only a few seconds. The slide was replaced into the test container on a microscope stage and the tail vessels brought into focus. The time intervals required for respiration and circulation, respectively, to stop after chilling and to start after warming, were measured by means of a stop-watch. The microscope and other equipment were set up in a constant temperature cold room set at the temperature of the test water. The temperature of the test water remained constant to within 0.2° C. during each experiment.

All data were analyzed statistically by a special method of computing chi square in an Rx2 table (Snedecor, 1956, p. 227).

Results

The guppy's normal temperature range, according to Innes (1955), is 16° C. to 40° C.; Gibson (1954), however limited the upper temperature to 32° C. When a guppy was exposed suddenly to water below 10° C., it exhibited the characteristics of "primary chill coma." Often after a few minutes at the low temperature, the fish's chromatophores expanded, causing the fish's color to darken. Exposures to cold temperatures above 10° C. resulted in "secondary chill coma."

Upon being replaced in a stock tank after "primary chill coma," the fish would usually begin to respire within eight minutes and almost invariably within twenty minutes, if they were to survive. Occasionally a fish would revive, show respiratory movements for several minutes to a few hours and then die. In "secondary chill coma," if a fish had gradually ceased to respire during the cold exposure, it usually did not revive when replaced in stock water of normal temperature, but if it was still respiring when taken from the cold, it usually survived.

I. Primary Chill Coma

A. Study of a single population

Approximately 700 guppies of all sizes were taken from a large aquatic plant tub in a University of Pennsylvania Botany Department greenhouse and acclimated to 23° C. ± 1° C. for 10 days. Batches of 10 adults, 5 males (80–130 mg.) and 5 females (100–900 mg.), and batches of 10 young guppies (6–18 mg.) were randomly picked and subjected to specific cold exposures. The rest of the fish were then acclimated to 30° C. ± 1° C. for 10 days; batches

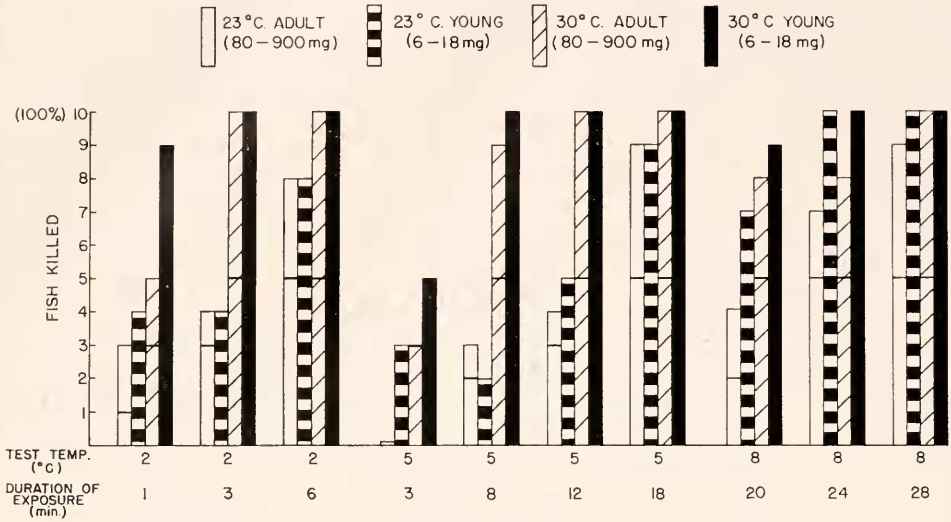

FIGURE 2. Death due to "primary chill coma" in the guppy. The black bar separates the females above from the males below.

of these adults and young were then subjected to the same cold exposures as those fish acclimated to 23° C. The results are summarized in Figures 2 and 5; Figure 2 shows the results of exposures to temperatures causing primary chill coma, and Figure 5 the results of exposures to temperatures causing secondary chill coma.

1. Effect of the temperature, duration of cold exposure, sex, size, and acclimation temperature on mortality

Combining all the data in Figure 2 it is seen that: (1) the lower the temperature, the more rapidly lethal effects occurred; (2) each increase in

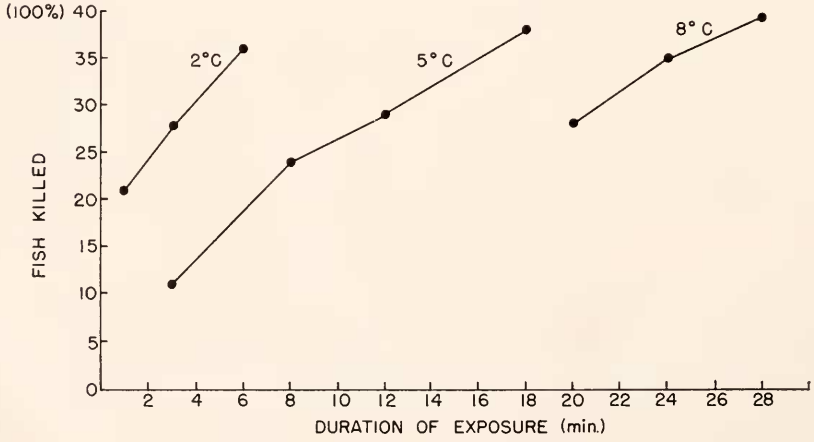

FIGURE 3. Total number of fish killed by each cold exposure at 2° C. ($p < 0.005$), 5° C. ($p << 0.001$), and 8° C. ($p < 0.005$).

the duration of exposure to a specific cold temperature was associated with an increase in the total number killed (Fig. 3); (3) males are less cold-tolerant than females ($p < 0.005$); (4) young are less cold-tolerant than adults ($p = 0.005$); (5) in both young ($p < 0.001$) and adults ($p < 0.001$) tolerance to "primary chill coma" is inversely related to acclimation temperature.

2. Effect of acclimation temperature on tolerance to anoxia at normal temperatures

When placed in 21° C. oxygen-depleted water (less than 0.25 mg. O_2/L.) for 15 minutes, none out of 20 fish acclimated to 23° C. succumbed, yet 10 out of 20 of fish acclimated to 30° C. died ($p = 0.001$). Tolerance to oxygen lack is thus inversely proportional to acclimation temperature.

Sumner and Doudoroff (1938), using boiled sea water and 0.001 molar potassium cyanide in sea water, likewise demonstrated that in the gobie, *Gillichthys mirabilis* Cooper, tolerance to anoxia was inversely related to acclimation temperature.

B. Lethal roles of the rapidness and repetition of chilling

1. Rapid versus slow chilling

In experiments where the water temperature was gradually reduced from the acclimation temperature to 2° C. in 15 minutes or to 8° C. in 7 minutes, 13 out of 16 fish died, whereas with rapid chilling to the same temperatures 13 out of 16 fish also died.

2. Lethal role of repetitive chilling

A batch of 20 adult guppies, 10 males and 10 females, acclimated to 23° C. was placed at 5° C. for one minute and subsequently at 23° C. for

TABLE I

Relationship of circulatory and respiratory functions in death due to "primary chill coma" in the guppy. Fish were acclimated to 29° C. for ten days

Fish No.		When chilled		When placed at 25° C.		Resp. started at	Condition 24 hrs. later
		Resp. stopped at	Circ. stopped at	Circ. started at	Circ. stopped at		
#1	5° C. for 3 min. ($\frac{1}{2}$ LD50)	less than 10 sec.	30 to 80 sec.				Alive
#2	5° C. for 3 min. ($\frac{1}{2}$ LD50)	less than 10 sec.	60 sec.				Alive
#3	5° C. for 5 min. (1 LD50)	3.6 sec.		15 sec.		75 sec.	Alive
#4	5° C. for 5 min. (1 LD50)	4.7 sec.		10 sec.		50 sec.	Alive
#5	2° C. for 3 min. (3 LD50)	2.3 sec.		15 sec.	2 to 11 min.	—	Dead
#6	2° C. for 10 min. (10 LD50)	3.5 sec.		210 sec.	7 to 10 min.	—	Dead
#7	2° C. for 65 min. (65 LD50)	2.6 sec.		—		—	Dead
#8	2° C. for 75 min. (75 LD50)	4.0 sec.		—		—	Dead

one minute. These exposures were repeated six times in succession. Each time the fish were chilled they lost all respiratory and swimming movement; but when they were placed at 23° C., most of them regained respiratory movements. None of these fish died.

C. Relationship of respiratory movements to the presence of a functioning circulation (Table I).

Careful individual observations were made on 30 fish subjected to 2° C., 5° C., or 8° C. Twenty fish received an LD50 exposure, 8 received 3 to 12 times the LD50 exposure, and 2 received over 60 times the LD50 exposure. In each of these 30 fish a complete cessation of all respiratory and swimming movements occurred within 10 seconds. The results were consistent; the data from 8 typical fish of this group are presented in Table I. The circulation, as judged by blood flow in the tail vessels of two fish (Fish #1 and #2, Table I), ceased approximately one minute after the fish were placed at 5° C.

If the heart beat returns it does so before there are any respiratory movements, as described by Britton (1924); in the present experiments if the circulation returned it did so before the respiratory movements, and the respiratory movements never returned unless a functioning circulation was already present.

Fish #3 and #4 (Table I) were subjected to an LD50 cold exposure and only transient respiratory and circulatory depression resulted. In all of the 20 fish subjected to an LD50 cold exposure the circulation returned. Respiration returned in 13 out of the 20 and they survived; in 4 others out of the 20, respiration returned for a brief period, subsequently stopped and the fish died; in the 3 others remaining out of the 20, respiration did not return and they died.

Fish #5 and #6 (Table I) were exposed to cold long enough to permit permanent respiratory depression but only transient circulatory depression. This resulted in the 8 fish exposed to 3 to 12 times the LD50 cold exposure as well as 3 of the 20 fish which received approximately an LD50 cold exposure.

Only 2 of the 30 fish (#7 and #8, Table I) received cold exposures of greater than 12 times the LD50 exposure. Neither the circulation nor the respiration returned in either fish and they of course did not survive.

D. Effect of increasing the tonicity of the chilling water

Fish were chilled at 5° C. for 8 minutes in distilled water, 0.32 molar sodium chloride (F.P. − 1.2° C.), or 0.65 molar glucose (F.P. − 1.2° C.) (the freezing point of teleost body fluid is between − 0.5° C. and − 0.9° C.; Brett, 1956). Increasing the tonicity of the chilling water exerted no significant effect on the guppy's cold tolerance, as shown by the fact that 21 out of 30 fish chilled in distilled water, 21 out of 30 fish chilled in 0.32 molar sodium chloride, and 20 out of 30 fish chilled in 0.65 molar glucose succumbed.

E. Effect of varying the gaseous content of the test water

1. Effect on mortality of varying the oxygen content and carbon dioxide content of the chilling water (Table II)

Increased $O_2 + CO_2$ or increased O_2 alone, during a cold exposure, lessened the lethality of the exposure (Exps. #1 and #3 Table II). Increased CO_2 alone had no such effect (Exp. #2 Table II). Exp. #4 (Table II) demonstrated that decreased O_2 during a cold exposure increases the lethality of the exposure.

TABLE II

Effect of increased oxygen and carbon dioxide (Exp. #1), increased carbon dioxide (Exp. #2), increased oxygen (Exp. #3), and decreased oxygen (Exp. #4) during cold exposures on tolerance to "primary chill coma"

	Treatment	Test water equilibrated with	O_2 content mg./L.	Number killed	Level of significance
Exp. #1	5° C. for 8 min.	Air	14.1	49 of 59 (83%)	$p \ll 0.001$
		95% O_2 + 5% CO_2	51.1	8 of 55 (15%)	
Exp. #2	5° C. for 10 min.	Air	14	39 of 40 (98%)	$p = 0.7$
		21% O_2 + 5% CO_2	14	38 of 40 (95%)	
Exp. #3	5° C. for 10 min.	Air	13.6	49 of 50 (98%)	$p \ll 0.001$
		100% O_2	51.5	31 of 46 (67%)	
Exp. #4	8° C. for 10 min.	Air	13.3	6 of 40 (15%)	$p = 0.02$
		100% N_2	<0.25	15 to 40 (38%)	

2. Effect on mortality of increased oxygen before, during, or after a cold exposure (Table III)

   Increased environmental oxygen during or after a cold exposure decreased the lethality of the exposure, but increased environmental oxygen before the exposure did not significantly alter this lethality (Table III). There is no significant difference ($p = 0.1$) between increased oxygen during or after a cold exposure (Batches #3 and #4, Table III).

3. Effect of increased oxygen on mortality when given during or after exposures to 2° C.

   Increased oxygen during one-minute cold exposures to 2° C. did not lessen mortality significantly ($p = 0.2$); 20 out of 30 young guppies (6–18 mg.) died when chilled in oxygen-depleted water (< 0.30 mg. O_2/L.), and 20 out of 40 died in O_2-enriched water (50 mg. O_2/L.).

   Twenty minutes in oxygen-enriched water after a three-minute exposure to 2° C. lessened mortality significantly ($p = 0.005$), for 24 out of 25 adult guppies died when replaced in a stock tank (10 mg. O_2/L.) while only 8

TABLE III

Effect of increased oxygen before, during, or after a cold exposure. Batches of 24 young fish (8 to 20 mg.) were chilled at 5° C. for 8 minutes. Batches #1, #2, and #4 were chilled in oxygen-depleted water (less than 0.30 mg. O_2/L.). Batch #3 was chilled in oxygen-enriched water (50 mg. O_2/L.). (Batch #2 was placed in oxygen-enriched water 40 mg.O_2/L.) for 20 minutes prior to the cold exposure and batch #4 for 20 minutes after the cold exposure.

Batch No.	Treatment	Per cent killed	p Value as compared to the control
1	CONTROL (Normal O_2 before and after)	100	
2	Increased O_2 BEFORE cold exposure	96	0.4[1]
3	Increased O_2 DURING cold exposure	21	<0.001
4	Increased O_2 AFTER cold exposure	46	0.001

out of 25 died when they were placed in oxygen-enriched water (40 mg. O_2/L.) of normal temperature prior to return to the stock tank.

F. Significance of anoxia and cold in causing death

1. Tolerance to anoxia at normal temperatures

Three randomly chosen batches of 10 adult guppies each, acclimated to $27 \pm 2°$ C., were placed in oxygen-depleted water at 26° C. (< 0.30 mg. O_2/L.) for different periods of time. Eight minutes of exposure killed none; 15 minutes killed 50%; and 20 minutes killed 100% ($p < 0.001$).

2. Interaction of cold and anoxia (Table IV)

There is a statistically significant difference in number killed between batch #3 and batches #1, #2, and #4 (Table IV). Nine minutes of anoxia prior to 5° C. for one minute (#3) killed significantly more than 5° C. for one minute alone (#1) ($p = 0.02$), 10 minutes of anoxia alone (#2) ($p = 0.005$), or 9 minutes of anoxia after 5° C. for one minute (#4) ($p = 0.05$). This indicated that anoxia before a cold exposure is more lethal than anoxia after a cold exposure.

TABLE IV

Interaction of cold and anoxia in producing death in "primary chill coma." Fish acclimated to 23 ± 1.0° C. for ten days

Batch No.	Treatment	Number killed
	Decreased oxygen (<0.25 mg./L.)	
1	5° C. for 1 min.	2 of 20 (10%)
2	25° C. for 10 min.	1 of 20 (5%)
3	25° C. for 9 min. followed by 5° C for 1 min.	9 of 20 (45%)
4	5° C. for 1 min. followed by 25° C. for 9 min.	3 of 20 (15%)
5	5° C. for 10 min.	18 of 20 (90%)
	Increased oxygen (50 mg./L.)	
6	5° C. for 10 min.	2 of 20 (10%)

When batch #5 (Table IV) was exposed to 5° C. for 10 minutes, a significantly higher number ($p = 0.005$) died than in batch #3 where fish were exposed to anoxia for 9 minutes and then to 5° C. for one minute. There is a highly significant difference between batch #5 and all the other batches. Batch #6 was exposed to 5° C. for 10 minutes in oxygen-enriched water (50 mg. O_2/L.); but only 2 out of 20 died as compared to 18 out of 20 in batch #5 which was exposed to 5° C. for 10 minutes in oxygen-depleted water (< 0.25 mg. O_2/L.) ($p \ll 0.001$).

G. Effect of prior anesthesia with 1% urethan (Fig. 4)

Prior anesthesia with 1% urethan decreases the lethal effect of "primary chill coma" ($p = 0.005$) (Fig. 4).

H. Effect of varying the sequence of two cold exposures (Table V)

Comparing batches #1 and #2 (Table V), a significantly higher number died ($p = 0.001$) among the fish exposed to the colder temperature first.

A suggestive decrease in mortality ($p = 0.1$) to an exposure to 2° C. for one minute occurred if the fish were first exposed to 8° C. for 4 minutes (Batches #2 and #3, Table V).

There is no statistically significant difference ($p = 0.25$) between 2° C. for

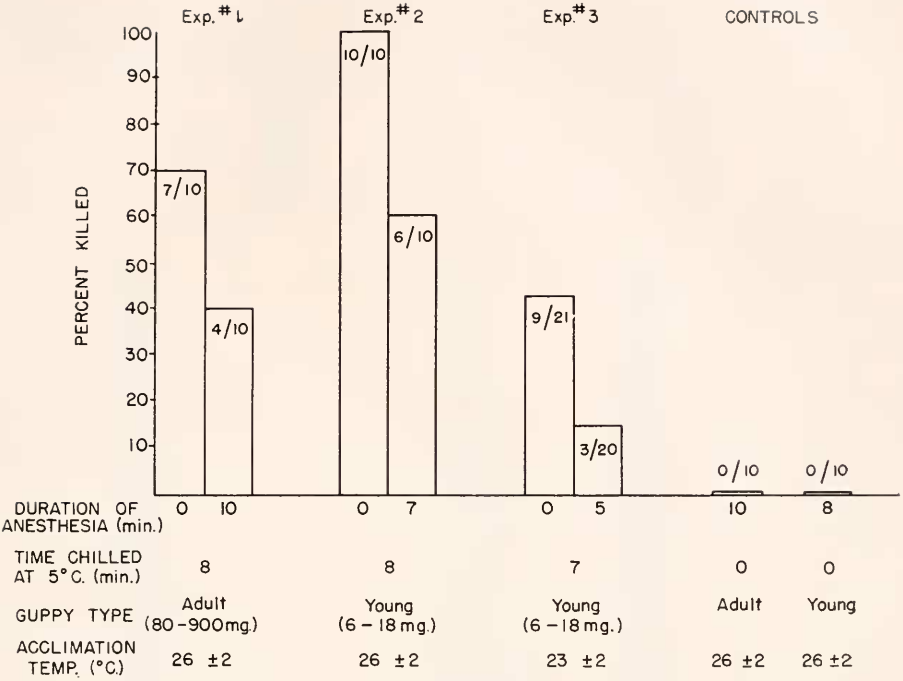

FIGURE 4. Protective effect of anesthesia with 1% urethan immediately before a cold exposure ($p = 0.005$).

5 minutes (Batch #4), and 2° C. for 1 minute when followed by 8° C. for 4 minutes (Batch #1).

There is a profound difference ($p \ll 0.001$) in lethal effect between 2° C. for 5 minutes (Batch #4), and 8° C. for 5 minutes (Batch #5).

II. Secondary Chill Coma

A. Study of a single population (Fig. 5)

1. Effect of the duration of a cold exposure on mortality

At 10.5° C., 20 out of 40 fish exposed for one hour died while 29 out of 40 exposed for one and one quarter hours died ($p = 0.05$). Only two

TABLE V

Effect of varying the sequence of two cold exposures. Three groups of ten adult guppies were used for each treatment

Batch No.	Treatment	Number killed
1	2° C. for 1 min. followed by 8° C. for 4 min.	25 of 30 (83%)
2	8° C. for 4 min. followed by 2° C. for 1 min.	12 of 30 (40%)
3	2° C. for 1 min.	19 of 30 (63%)
4	2° C. for 5 min.	30 of 30 (100%)
5	8° C. for 5 min.	2 of 30 (7%)

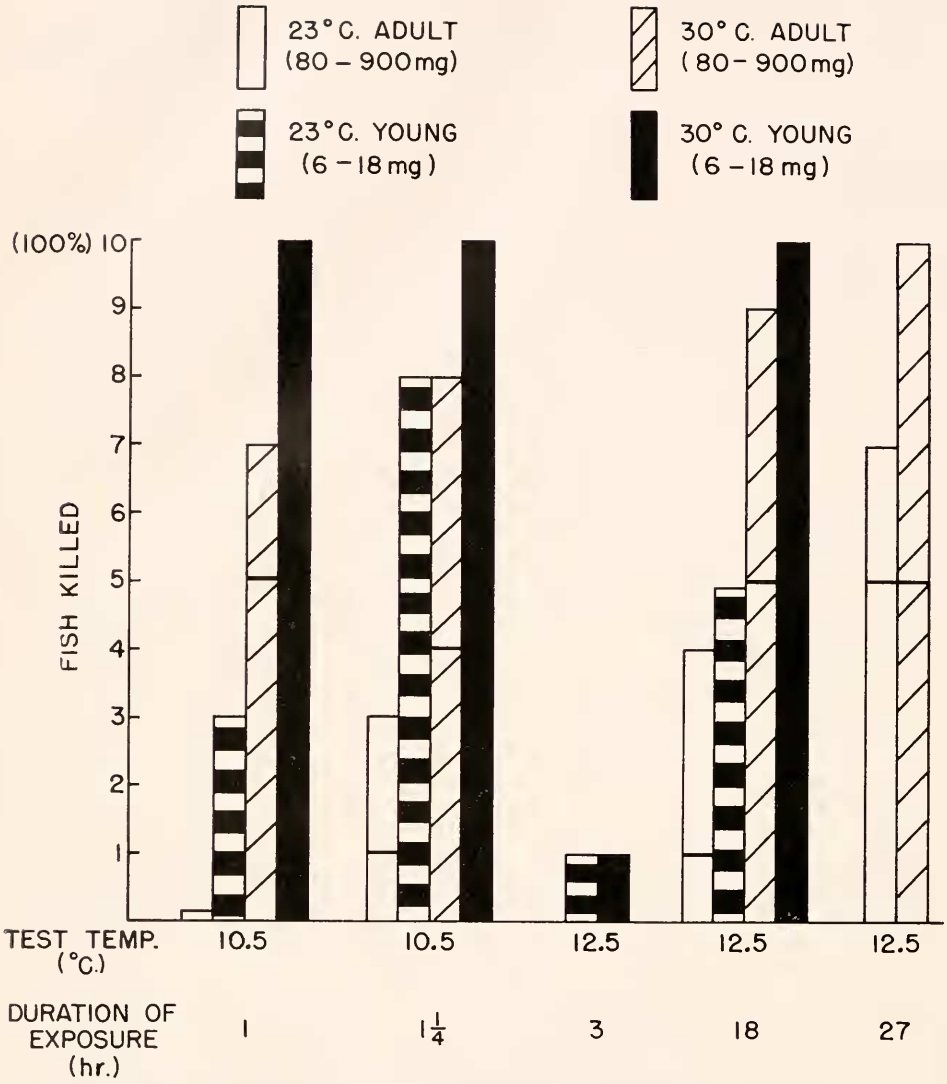

FIGURE 5. Death due to "secondary chill coma" in the guppy. The black bar separates females above from males below.

out of 20 young guppies exposed to 12.5° C. for 3 hours died while 15 out of 20 exposed for 18 hours died ($p < 0.001$). Thirteen out of 20 adults exposed to 12.5° C. for 18 hours died while 17 out of 20 exposed for 27 hours died ($p = 0.25$). (This last comparison is the only one of the three where the difference is not statistically significant.) These data indicate that in "secondary chill coma," as in "primary chill coma," the duration of exposure determines the mortality.

TABLE VI

Effect of increased oxygen content of the chilling water on tolerance to "secondary chill coma." Fish acclimated to 27 ± 2° C. Combining the results from the three experiments, $p = 1.0$

	Treatment	Number killed	
		10 mg. O_2/L.	45 mg. O_2/L.
Adult fish (100–800 mg.)			
Exp. #1	11.4 ± 0.3° C. for 15.5 hrs.	24 of 25 (96%)	25 of 25 (100%)
Exp. #2	12.1 ± 0.2° C. for 8.0 hrs.	15 of 20 (75%)	15 of 20 (75%)
Juvenile fish (20–40 mg.)			
Exp. #3	12.1 ± 0.2° C. for 12.0 hrs.	20 of 25 (80%)	19 of 25 (76%)

2. Effect of sex, size, and acclimation temperature on mortality

Comparing data in Figure 5, males do not seem less cold-tolerant than females ($p = 0.3$); but young are much less cold-tolerant than adults ($p = 0.005$). Cold-tolerance is inversely related to the acclimation temperature in both young ($p < 0.001$) and adults ($p = 0.005$).

B. Effect of increased oxygen during the cold exposure (Table VI)

Increased oxygen during secondary chill coma did not lessen mortality (Table VI).

C. Effect of isosmotic chilling medium on mortality

Two batches of young guppies (8–18 mg.) were subjected to 12.0° C. for 5.5 hours. Batch #1 was chilled in distilled water and batch #2 in 0.16 molar sodium chloride (F.P. − 0.6° C. This is approximately isotonic with teleost body fluids (Brett, 1956). Nine out of 23 fish in batch #1 succumbed; 10 out of 24 in batch #2 died. There is no significant difference between these two batches ($p = 0.75$).

DISCUSSION

Among the possible causes of death inherent in cold exposure, two factors may be excluded. The suddeness of a cold exposure is not of itself lethal, for sudden exposures did not cause more mortality than gradual exposures (IB1)[3] (Britton, 1924). Moreover, the cooling process *per se* is not lethal since even repetitive chilling into "primary chill coma" caused no mortality (IB2). At a specific cold temperature, the duration of cold exposure is the decisive determinant of lethality rather than the abruptness or repetition of the temperature change.

"Primary Chill Coma"

Taken altogether, the data suggest that "primary chill coma" kills by causing anoxic damage to a cold-depressed respiratory center. The permanent re-establishment of respiration after "primary chill coma" is the critical event in determining survival (IC). Even after prolonged lethal exposures to cold, the fish's circulation often returns within a few minutes (IC) and then, depending on one major variable, permanent respiration does or does not return. This variable is the

[3] This refers to pertinent experimental data listed under results.

amount of oxygen which the circulation can bring to the respiratory center to forestall anoxia damage while the cold depression of respiration subsides.

There appear to be several factors limiting the availability of oxygen. Sumner and Doudoroff (1938) showed that fish have a considerable oxygen reserve in their tissues. Consequently, in the present experiments the relative depletion or saturation of the fish's oxygen reserve partially determines the amount of oxygen that the circulation can carry to the fish's respiratory center. Normally the oxygen reserve is probably maximal, since increased oxygen before "primary chill coma" did not lessen mortality (IE2). If the oxygen reserve had been submaximal, then the extra oxygen should have diffused into the fish's superficial tissues (Privol'nev, 1956; Breder, 1941) and protected the animal against anoxia. Any process which depletes the oxygen reserve increases mortality. The oxygen reserve may be depleted by a prior anoxic period (IF2), decreased environmental oxygen during a cold exposure (IE1), or the extra oxygen consumption which accompanies the initial convulsive paroxysm. Anesthesia prior to a cold exposure lowered mortality (IG), probably by lessening the initial convulsive paroxysm and so lessening this initial depletion of the oxygen reserve.

The protective value of increased oxygen during and after chilling (IE) may be explained by the hypothesis that when the fish's oxygen reserve is depleted, more oxygen can diffuse into the fish and replenish that portion of the depleted oxygen reserve located in the cutaneous and subcutaneous tissues. Ultimately the oxygen probably protects by acting centrally. Nervous tissue is the most sensitive to anoxia of any vital tissue, and so it is probable that the increased oxygen benefits the respiratory center directly. This effect of increased environmental oxygen in promoting respiratory return and survival does not seem to be related to any reflex mechanism for at least two reasons: (1) Increased environmental oxygen would lessen rather than increase reflex stimulation of the respiratory center; (2) increased oxygen protected even when given only during the cold exposure and not when respiration actually restarted (IE).

The observation that four of the thirty individually observed fish died after respiration was re-established (IC) may have either of two alternative explanations: (1) There is an additional mechanism or mechanisms for death due to primary chill coma; (2) when rewarmed the cold depression of the respiratory center was relieved and it began functioning while still anoxic; this rendered it more anoxic and permitted irreversible damage to occur.

In experiments where oxygen was unaltered, mortality was increased by lowering the temperature of a cold exposure of a specific duration (IA1) (IH) and also by subjecting fish first to the lower of two sequential cold exposures (IH). The lower chilling temperature probably induces a more profound respiratory depression and a more vigorous initial convulsive paroxysm which more completely depletes the oxygen reserve.

Tolerance to "primary chill coma" is inversely related to acclimation temperature (IA1). Perhaps a higher acclimation temperature lessens the fish's oxygen reserve since there is less oxygen dissolved in body water of higher temperatures; however, this is partially offset by an increase in diffusion (Krogh, 1919). A higher acclimation temperature increases the overall metabolic rate so that the fish's oxygen reserve is depleted more rapidly. With an increase in the acclimation temperature

the central nervous system would have an increased oxygen need (Freeman, 1950) and the respiratory center would become anoxic more rapidly.

Oxygen may be important also in the observations that young are less tolerant to "primary chill coma" than adults (IA1) and males are less tolerant than females (IA1). These less tolerant groups are smaller, have a proportionately higher metabolic rate (Muller, 1942), and so upon chilling might become anoxic more rapidly. Perhaps they would experience also a more profound respiratory depression, for their smaller size would permit the cold to penetrate more rapidly to the respiratory center. Their smaller size should permit a more rapid replenishment of oxygen reserve via cutaneous diffusion, but evidently this advantage is overshadowed by the higher metabolic rate and perhaps by the more rapid penetration of the cold in the smaller fish. It is possible that young guppies and or male guppies may have a respiratory center which is innately more susceptible to cold depression or anoxic damage or both.

"Secondary Chill Coma"

The experimental data presented in this paper give no indication of the cause of death due to "secondary chill coma." Osmoregulative failure (Doudoroff, 1945) does not seem to occur in the guppy, for chilling in isosmotic sodium chloride had no protective effect (IIC). Although increased oxygen protects against "primary chill coma," it does not protect against "secondary chill coma" (IIB).

Certain observations are perhaps significant. Fish acclimated to higher temperatures are more sensitive to "secondary chill coma" (IIA2); young are more sensitive than adults (IIA2). These more sensitive groups have a significantly higher metabolic rate and would exhaust their energy stores more rapidly so that death due to "secondary chill coma" might be some sort of exhaustion phenomenon. There does not seem to be a sexual difference in sensitivity to "secondary chill coma." The difference in metabolic rate between male and female guppies is perhaps not enough to produce significant differences in the limited number of fish used.

Despite the present inability to explain the mechanism or mechanisms of death due to "secondary chill coma," all the data that have been obtained to the present time can be reconciled with the proposition that death due to "primary chill coma" is caused by anoxic damage to a cold-depressed respiratory center.

I am indebted to the late Dr. L. V. Heilbrunn for first stimulating my interest in this problem and for providing laboratory space. I wish to thank Dr. John R. Brobeck for sponsoring this project and for his many valuable suggestions and encouragement, and Dr. Harold T. Hammel for his advice and aid.

Summary

A. Observations concerning both "primary chill coma" and "secondary chill coma"

1. In guppies acclimated to 23 to 30° C., exposures to temperatures below 10° C. produced "primary chill coma" while exposures to lethal temperatures above 10° C. caused "secondary chill coma."

2. The duration of a cold exposure at a specific temperature is the decisive lethal

determinant rather than the chilling temperature *per se*. An increase in the duration of a cold exposure causes an increase in mortality.

3. Tolerance to a cold exposure is inversely related to acclimation temperature. Tolerance to oxygen lack at normal temperatures also is inversely related to acclimation temperature.

4. Males are less cold-tolerant than females in the temperature range of "primary chill coma." There does not seem to be a sexual difference in cold tolerance in the temperature range of "secondary chill coma."

5. Young guppies (6–18 mg.) are less cold-tolerant than adults (80–900 mg.).

B. Observations on "primary chill coma"

1. Respiration did not return in any fish subjected to approximately three or more times the LD50 cold exposure, despite the fact that the circulation returned in all fish subjected to approximately twelve times the LD50 exposure or less.

2. The lethality of a cold exposure was increased by decreased oxygen before or during the exposure.

3. The lethality of a cold exposure was decreased by prior anesthesia with 1% urethan, increased oxygen during or after the exposure, or with two sequential cold exposures by exposing the fish to the less extreme temperature first.

4. The lethality of a cold exposure was unaltered by increased oxygen before the exposure, decreased oxygen (of a duration which was not lethal of itself) after the exposure, increased tonicity of the chilling water, or equilibrating the chilling water with 5% CO_2.

C. Observations on "secondary chill coma"

1. The lethality of a cold exposure was unaltered by increased oxygen during the exposure, or by the use of isotonic sodium chloride as the chilling medium.

D. The following conclusions may be drawn from the observations made:

1. Death due to "primary chill coma" in the guppy may be due to anoxic damage to a cold-depressed respiratory center.

2. The lethal effect of "primary chill coma" is related to the profoundness of respiratory depression and by the degree of depletion of the fish's oxygen reserve.

3. Osmoregulative failure does not seem to be a cause of death in "secondary chill coma" in the guppy.

4. Oxygen lack is not a lethal determinant in "secondary chill coma" in the guppy.

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